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METHODS PAPER

High throughput estimation of incident light, light interception and radiation-use efficiency of thousands of plants in a phenotyping platform

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Summary

- Light interception and radiation use efficiency (RUE) are essential components of plant performance. Their genetic dissections require novel high-throughput phenotyping methods.
- We have developed a suite of methods to evaluate (i) the spatial distribution of incident light as experienced by hundreds of plants in a greenhouse, by simulating sun beam trajectories through greenhouse structures every day of the year (ii) the amount of light intercepted by maize (*Zea mays*) plants, via a functional-structural model using 3D reconstructions of each plant placed in a virtual scene reproducing the canopy in the greenhouse and (iii) RUE, as the ratio of plant biomass to intercepted light.
- The spatial variation of direct and diffuse incident light in the greenhouse (up to 24%) was correctly predicted at the single-plant scale. Light interception largely varied between maize lines that differed on leaf angles (nearly stable between experiments) and area (highly variable between experiments). Estimated RUEs varied between maize lines but were similar in two experiments with contrasting incident light. They closely correlated with measured gas exchanges.
- The methods proposed here identified reproducible traits that might be used in further field studies, thereby opening the way for large-scale genetic analyses of the components of plant performance.

Key words: environmental characterization, high-throughput phenotyping, maize, light interception, radiation-use efficiency, architecture

Introduction

Understanding the genetic controls of biomass production and yield is a major challenge in the context of climate change (Murchie *et al.*, 2009; Zhu *et al.*, 2010; Reynolds *et al.*, 2012). Yield (Y) can be dissected as a function of incident light (PPFD), the fraction of light intercepted by the crop (ϵ), the efficiency of the conversion of light into biomass, also called radiation-use efficiency (RUE,(Monteith, 1977)) and the partitioning of biomass to yield (harvest index, HI):

$$Y = HI \times \sum_{i=1}^n PPFD_i \times \epsilon_i \times RUE_i, \quad (1)$$

where n is the duration of crop growth (d), $PPFD_i$, ϵ_i and RUE_i are the incident light, the fraction of intercepted light and RUE on the i^{th} day. Y can be genetically improved by increasing any of the terms of Eq. 1. Whereas HI has been one of the main determinants for improving yields in wheat during the 20th century (Calderini *et al.*, 1995; Sayre *et al.*, 1997), there is probably little avenue for further improvements in most crops (Austin *et al.*, 1980; Foulkes *et al.*, 2011; Reynolds *et al.*, 2012). The remaining terms of Eq. 1, ϵ and RUE, are directly related to light capture and photosynthetic efficiency at canopy level (Zhu *et al.*, 2010; Reynolds *et al.*, 2012). The genetic variability of leaf area development has a high effect on light interception at early stages of the plant cycle (Hay & Porter, 2006; Murchie *et al.*, 2009). Changes in canopy architecture also affect interception *via* genotypes with erect leaves that decrease light saturation at the top of the canopy and allow better penetration of light, thereby reducing the proportion of leaf area experiencing low light (Long *et al.*, 2006; Zhu *et al.*, 2010; Reynolds *et al.*, 2012). Studies comparing the relationship between the genetic variabilities of leaf architecture and light interception at the intraspecific level have been limited to a small number of genotypes, with contrasting conclusions (Louarn *et al.*, 2008; Hammer *et al.*, 2009; Moreau *et al.*, 2012).

Estimations of RUE are usually based on consecutive and destructive sampling of aboveground biomass over time, which is not feasible for the large number of genotypes involved in genetic analyses (Sinclair & Muchow, 1999). Gas-exchange measurements are also difficult to tackle at high throughput and are usually limited to measurements at the single-leaf level (Albrizio & Steduto, 2005). A phenotyping platform allowing measurements of 3D plant architecture and estimates of plant biomass with a time definition of one day

offers new possibilities to estimate light interception and RUE of hundreds of genotypes, together with their responses to environmental conditions. However, no method is currently proposed because of technical difficulties.

- The spatial variability of incident light can be up to 30% within a greenhouse (Stanhill *et al.*, 1973; Kozai & Kimura, 1977; Brien *et al.*, 2013) or a growth chamber (Granier *et al.*, 2006). Conditions also vary between experiments, in greenhouses because of climatic conditions and in growth chambers because of differences between chambers (Massonnet *et al.*, 2010). A genetic analysis of plant performance therefore requires a precise evaluation of the PPFD (diffuse and direct) available to each plant of the greenhouse or growth chamber during each experiment.

- Light interception can be derived from 3D plant architecture, combined with estimates of the direction of sunbeams and with the proportion of direct vs. diffuse light (Sinoquet *et al.*, 2001). Platform experiments present a difficulty compared with the field, namely that they often harbour composite canopies in which each plant is surrounded by plants of another genotype. Hence, it is necessary to distinguish the light interception by each plant to dissect the genetic variabilities of ϵ and RUE.

The objective of this paper was to develop a non-invasive, automatized and accurate procedure to derive light interception and radiation-use efficiency in high-throughput phenotyping platforms. To our knowledge, we present here the first methods for estimating the local PPFD received by each individual plant and for estimating light interception and RUE at a throughput of thousands of plants. We have tested whether RUE derived from the methods presented here is stable between experiments and related to leaf gas-exchange measurements.

100 ***The PHENOARCH phenotyping platform***

101 The PHENOARCH platform (<http://bioweb.supagro.inra.fr/phenoarch>), hosted at the M3P,
 102 Montpellier Plant Phenotyping Platforms (<https://www6.montpellier.inra.fr/lepse/M3P>), is
 103 based on a PhenoWare™ system (PhenoWare™, Lyon, France) composed of a conveyor belt
 104 structure of 28 lanes carrying 60 carts with one pot each (i.e. 1680 pots), plus a conveyor
 105 belt system that feeds the imaging or the watering units. The imaging unit is composed of
 106 two cabins with 3D image acquisition involving top and side RGB cameras (Grasshopper3,
 107 Point Grey Research, Richmond, BC, Canada) equipped with 12.5-75mm TV zoom lens
 108 (Pentax, Ricoh Imaging, France) and LED illumination (5050 - 6500K colour temperature).
 109 Five watering units are composed of weighing terminals (ST-Ex, Bizerba, Balingen, Germany)
 110 and high-precision pumps (520U, Watson Marlow, Wilmington, MA, USA). Circulation of
 111 plants through conveyors, image acquisition and irrigation management are controlled by an
 112 industrial open automation system based on PC Control technology (Beckhoff CX 2020,
 113 Beckhoff Automation, Verl, Germany) that allows localization in real time of every pot in the
 114 platform and individually programming pot displacements. Imaging and watering routines
 115 are sequentially performed every day. Plants are then moved back to the same positions and
 116 orientation, so plant position in respect to neighbours is conserved throughout the
 117 experiment. PHENOARCH has held experiments with different species including cereals
 118 (maize (*Zea mays* L.), wheat, rice, sorghum) (Sciara *et al.*, 2015), grapevine (Coupel-Ledru *et*
 119 *al.*, 2014) and apple trees (Lopez *et al.*, 2015). The plant density can be adapted for each
 120 species, from 13 plants m⁻² in the default setting with 1680 plants to double densities for
 121 smaller plants or half densities for small trees or adult maize plants. Experiments performed
 122 until mid-2014 used a Lemnatec technology (LemnaTec, Wüerselen, Germany) that was then
 123 replaced by the methods presented above.

124 Micro-meteorological conditions are constantly monitored at six positions in the greenhouse
 125 at the top of the plant canopy. Air temperature and humidity are measured every minute
 126 (HMP45C, Vaisala Oy, Helsinki, Finland), together with PPFD (SKP215, Skye Instruments,
 127 Powys, UK). The temperature of the meristematic zone of eight plants distributed in the
 128 greenhouse is measured with a fine copper-constantan thermocouple (0.2 mm diameter)
 129 located between the sheaths of two leaves located at meristem height. Air vapour pressure

difference (VPD) is estimated at each time step as the difference in water vapour pressure between saturation at air temperature and the current vapour pressure in the air. All data of air/meristem temperature, PPFD and relative humidity are averaged and recorded every 15 min (NI CompactRio, National Instruments, Austin, TX, USA) and stored in the PHIS database (<http://web.supagro.inra.fr/phis>).

Spatial variability of incident light in the greenhouse

Daily incident PPFD over each plant of the platform ($PPFD_{(xy)}$) was estimated by combining a 2D map of light transmission and the outside PPFD ($PPFD_{ext}$) measured every 15 min with a sensor placed on the greenhouse roof (SKS 1110, Skye Instruments, Powys, UK). Maps of the fraction of transmitted direct (T_{dir}) and diffuse (T_{dif}) light were calculated every hour of each day of the year by using 169 hemispherical images of the greenhouse using a digital camera (Nikon Coolpix 4500, Nikon, Melville, NY, USA) fitted with a fisheye lens with a 183° field of view (Nikon FC-E8 Fisheye Converter, Nikon). Images were taken every m² in the greenhouse, at 0.4, 1 and 1.5 m high, i.e. at heights representing the top of canopies of different species and phenological stages. Only data at 1m height are presented here. The camera and lens were placed vertically (checked with a spirit level) and the geographical North was referenced. Hemispherical images were analysed using the Ilastik 1.1.8 software (Sommer *et al.*, 2011) (Supporting Information Fig. S1, Table S1). Calculation of sun paths, transmitted direct and diffuse radiation was then performed using standard astronomical formulae using R scripts (R_Core_Team, 2015) available as an open application (Supporting Information Methods S1). The refraction in the glass of the greenhouse changed the angle of sun beams by 0 to 36° for incident angles up to 60°. It returned to its original value in air so refraction resulted in a translation of sunbeams by 0 to 2 cm, and was therefore considered as negligible. The amount of transmitted direct radiation was computed every hour as a function of solar position, calculated from the daily time-course of sun path, in relation to the gap fraction at each position along the sun path (i.e. fraction of the image without greenhouse structure or lamps, Fig. 1). The amount of transmitted diffuse PPFD was calculated using a standard overcast sky (SOC) diffuse model (Moon & Spencer, 1942), in which diffuse radiation flux varies with zenith angle and then depends on the gap fraction of the greenhouse. Transmissions of direct and diffuse light were further corrected by the

transmittance coefficient of light through glass, measured using a spectroradiometer (HR4000, Ocean Optics, Dunedin, FL, USA).

The amount of PPFD reaching each of the XY positions within the greenhouse ($PPFD_{(x,y)}$) on a given day (i) was calculated as:

$$PPFD_{(x,y)i} = \sum_{j=0}^d f_{d,j} \times PPFD_{ext,j} \times T_{dir(x,y)j} + (1 - f_{d,j}) \times PPFD_{ext,j} \times T_{dif(x,y)} \quad (2)$$

Where f_d is the fraction of direct light and $(1 - f_d)$ is the fraction of diffuse light from incoming external global radiation corresponding to the j^{th} hour and d the duration of daylight, calculated according equations detailed in (Spitters *et al.*, 1986).

Image analysis and reconstruction of plant architecture

RGB colour images (2056 x 2454) from thirteen views (twelve side views from 30° rotational difference and one top view) were captured daily for each plant during the night. Images were captured while the plant was slowly rotating using a brushless motor. Top and side cameras were calibrated using reference objects in order to convert pixels into mm^2 . Plant pixels from each image were segmented from those of the background with HSV thresholding using OpenCV libraries (Open Source Computer Vision Library: <http://opencv.org>). A 3D representation of each plant of the platform was obtained using a silhouette-carving algorithm. Plant skeletons were extracted from binarised 2D RGB images using the thinning algorithm of (Zhang & Suen, 1984), implemented in ImageJ (Rasband, 1997-2014). Skeletons were further processed with the 'Analyse Skeleton' ImageJ plugin (Arganda-Carreras *et al.*, 2010) to obtain crossings and the endpoints of the different branches of the skeleton. These points were used to navigate through the skeleton image and segment it into 50-pixels-long elementary lines. The angle of each elementary segment with the vertical was computed as the absolute value of the arctangent between z- and x-coordinates of segments endpoints. All data, namely raw and processed images together with metadata were stored in the PHIS database (<http://web.supagro.inra.fr/phiss/>).

Light interception and radiation-use efficiency

Daily light interception was estimated for each plant of the platform by using the functional-structural RATP (radiation absorption, transpiration and photosynthesis) plant model (Sinoquet *et al.*, 2001) available under OpenAlea platform (<http://openalea.gforge.inria.fr/dokuwiki/doku.php>) (Pradal *et al.*, 2008; Pradal *et al.*, 2015).

Briefly, the canopy was split into cubic voxels of 0.2 m, characterized each by the density of leaf area and the leaf angle distribution calculated from the 3D virtual representations of the neighbouring plants. Density of leaf area was calculated as the cumulative area of all leaf segments in the voxel, regardless of the plant they originated from. The calculated mean leaf angle in a voxel was calculated as the mean of angles of all leaf segments in the considered voxel.

For each voxel, intercepted PPFD was calculated every day from a sample of 46 beam angles, with a cumulative value equal to the incident PPFD at the corresponding x y position in the greenhouse (PPFD_(xy)). For each direction, beam extinction was computed by applying Beer's law within the sequence of intersected cells by each beam. The daily PPFD intercepted by each plant was obtained by cumulating the PPFD interception for each voxel weighed by the relative contribution of the considered plant to the voxel area. Radiation-use efficiency was then estimated as the slope of plant biomass production to cumulative intercepted PPFD.

Leaf gas exchange measurements

A portable open gas exchange system (LI-COR 6400XT, LI-COR Inc., Lincoln, NE, USA) was used to measure leaf gas exchange in youngest fully expanded leaf blades in a set of eight maize lines. The net CO₂ assimilation rate (A_N), stomatal conductance (g_s) of those leaves were measured inside the greenhouse from 10:00 to 14:00 (solar time) at 1500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ of PPFD, a leaf temperature of 28°C, a leaf-to-air vapour pressure deficit of about 1.2-1.5 kPa and an ambient CO₂ of 400 $\mu\text{mol mol}^{-1}$.

Plant growth

The leaf area and the fresh plant weight of individual plants were estimated from images taken in 13 directions. Briefly, pixels extracted from RGB images were converted into fresh plant weight and leaf area using linear models derived from regression of data from multiple side view images and destructive measurements performed at different phenological stages, from 5 to 14 appeared leaves (i.e. from 15 to 50 days at 20°C after emergence). The resulting conversion was accurate and unbiased (Supporting Information Fig. S2). The time courses of leaf area or fresh plant weight were then fitted individually to the three-parameter Gompertz function,

$$y = a \times e^{-e^{(b-cx)}} \quad (3)$$

using appropriate R scripts (R_Core_Team, 2015). Time courses were expressed as a function of equivalent days at 20°C (Parent *et al.*, 2010). At the end of the experiment, shoots of all plants were harvested and total plant biomass was measured.

Genetic material and growth conditions

The techniques presented above were tested in two experiments carried out in autumn (Exp. 1) and winter-spring (Exp. 2), with markedly different incident PPFD. Two panels were studied involving 60 and 200 maize (*Zea mays* L.) lines for Exp. 1 and Exp. 2, respectively. A common set of 23 maize inbred lines with tropical origin was grown in both experiments, chosen for maximising the genetic and phenotypic variabilities. Plants were grown in polyvinyl chloride (PVC) 9 L pots (0.19 m diameter, 0.4 m high) filled with a 30:70 (v/v) mixture of a clay and organic compost. Three seeds per pot were sown at 0.025m depth and thinned to one per pot when leaf three emerged. In each of the experiments two levels of soil water content were experienced; (i) retention capacity (WW, soil water potential of -0.05 MPa) and (ii) mild water deficit (WD, soil water potential of -0.5 MPa) by compensating transpired water three times per day via individual measurements of each plant. The weight of water in each pot was calculated at the beginning of the experiment from the weight of soil and measured soil water content. It was then maintained at a constant value by considering that the weight loss between two time-points was due to transpiration plus soil evaporation, after correction for the change in plant fresh weight every day (Eq. 3). Each line was replicated 5 and 7 times for the WW and WD treatments, respectively in Exp. 1, whereas each line was replicate 4 times in Exp. 2. Greenhouse temperature was maintained at 25 ± 3°C during the day and 20°C during the night. Supplemental light was provided either during day time when external solar radiation dropped below 300 W m⁻² or to extend photoperiod using 400 W HPS Plantastar lamps (OSRAM, Munich, Germany) with 0.4 lamps m⁻². The resulting photoperiod was 12/12h day/night. The amount of light supplied by lamps was taken into account in the calculations of local PPFD.

Statistical analyses

Two-way analyses of variance (ANOVA) were performed using the lm procedure (R_Core_Team, 2015) to calculate the effects of experiment and genotype. Broad-sense heritability (h^2) was calculated as:

$$h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_e/r) \quad (4)$$

Where σ^2_g is the genotypic variance, σ^2_e is the residual variance, and r is the number of plant replicates per genotype. All statistical tests and graphs were performed using R 3.1.3 (R_Core_Team, 2015).

Results

Estimating the spatial variability of local available light for each plant of the platform

We have modelled the fraction of direct and diffuse light reaching each plant every hour of each day of the year based on 169 hemispherical images taken at different x-y positions of the greenhouse (1 image m^{-2}), which capture the obstacles to light (e.g. beams of the greenhouse, lamps or cabins, Fig. 1). For each image, the daily time-course of sun path was simulated based on the latitude, day of year and time of day, as presented in Fig. 1a-d for summer and winter solstices and spring and autumn equinoxes. Sun paths occupied more central positions in the image and were longer during summer compared to winter because of the changes of solar position with the vertical. They crossed the structures of the greenhouse for a fraction of the day and reached plants (path superimposed on the sky) otherwise. The proportion of transmitted direct PPFD was estimated from the gap fraction over the sun path (frequency for a light beam to cross the structure in the absence of obstacle), weighed for light intensity and displacement rate along the sun path at each time-step (Supporting Information, Methods S1). Light transmission through the greenhouse glass was also estimated, resulting in a transmittance coefficient (k_g) of 0.76.

Daily transmission of direct light was calculated every day for each of the 1680 positions in the greenhouse, resulting in large spatial and temporal variations. The duration of peaks of transmission was shorter in winter than in summer solstices, whereas the maximum value was close to 60% in all cases (Fig. 1e-h). The time course of transmission also changed with position in the greenhouse (Fig. 1e-l), with a spatial variability that was greater in winter, with up to 30% differences between locations, compared to 14% in summer. The x-y position with maximum transmission itself changed with time of year. Integrated over one day, these data resulted in a map of direct transmission for each day of the year (Fig. 2i-l, Supporting Information Video S1). The transmission of diffuse radiation was also calculated for each position of the greenhouse from the same hemispherical images, resulting in a spatial variation of 11.9% (Fig. 2b). Whereas transmission of direct light maps was calculated each

day of the year (Fig. 2a), the transmission of diffuse light only depends of the greenhouse structure so the map presented in Fig 2b is independent of days of the year. The total amount of direct plus diffuse PPFD reaching each location in the greenhouse was finally estimated using Eq. 2 (Fig. 2).

The method presented above can be extended to any other greenhouse provided that images are available (Supplementary Information Methods S1). It was tested by comparing the simulated PPFD at six locations in the greenhouse with PPFD measured at the same locations. Measured PPFD cumulated over one week showed an appreciable spatial variability (24%, from 173 to 220 mol m⁻²), which was adequately accounted for by the simulated PPFD at the same positions (Fig. 3a). The daily time courses of observed vs simulated PPFD averaged over the six positions in the greenhouse were also compared on two days with high and low PPFD values, respectively. Fig. 3b shows that observed and simulated time courses closely matched, so the cumulative PPFD values estimated with the methods presented above was not biased.

Estimating leaf angles over time

The side plant image containing maximum information for the quantification of leaf angles was chosen for each plant and day as that containing most leaves (Fig. 4a). To that end, we have used the binarised top view of the plant on which we performed a reduced major axis regression, allowing us to choose the side image with the best angle (Fig. 4b). This image was then segmented and skeletonized (Fig. 4cd). The endpoints of the different branches of plant skeletons (red circles) were used to navigate through the skeleton and to dissect it into 50-pixels-long elementary segments (Fig. 4f). The orientation of each elementary segment was then computed, thereby allowing calculation of angles for each segment. The mean leaf angle was then computed either for a whole plant or in each voxel, as shown in Fig. 4e for the plant presented in Fig 4a-e. An example of this procedure is shown in Figure 4g where mean leaf angles clearly differed between three maize lines showing either sub-horizontal angles (close to 0) or more erect architectures. Angles corresponding to each line remained essentially stable with phenological stages as illustrated in Figure 4h.

Calculating daily light interception by each plant of the greenhouse

The composite canopy in the greenhouse (i.e. with neighbouring plants belonging to different lines) was reconstructed from individual 3D point cloud representations of each

plant, and split into in a grid of voxels for calculating light interception using the RATP model. For each plant, actual plant leaf area and plant leaf angle distribution were uniformly distributed among the 3D point cloud. Figure 5 represents a schematic diagram of how the RATP modelling inputs were generated:

- First, reconstructed 3D plants were positioned according to their actual coordinates in the greenhouse to obtain a 3D point cloud representing the canopy (Fig 5a).
- Second, a 3D grid with cubic voxels of 0.20 m side was fit to the canopy bounding box and filled using positions, leaf angles and leaf areas associated to each point of the 3D canopy (Fig. 5a). Each voxel may thus include leaves of several neighbouring plants, with a range of angular distribution. Fig. 5b represents the whole greenhouse discretized in this way, where represented volumes are proportional to the leaf area in the corresponding voxel and the colour represents the dominant leaf angle.
- Fourth, intercepted PPFD was calculated every day in each voxel from local $PPFD_{(xy)i}$ obtained with light maps. The calculation began with upper voxels, so the light reaching voxels at lower positions depended on both local PPFD and on transmission through upper voxels. The amount of PPFD intercepted by each voxel was then partitioned between neighbouring plants as a proportion of leaf area of each plant in the considered voxel.
- Finally, the daily PPFD intercepted by each plant was computed by cumulating PPFD intercepted by this plant by each voxel.

An example of reconstructed architecture of ten neighbouring plants and of calculated intercepted PPFD is presented in Fig. 6 at three times of the plant cycle (20, 35 and 50 d after sowing). Leaves increasingly interacted with each other on days 35 onwards (Fig. 6a-c). Calculated intercepted light (Fig 6d-f) increased with time, with an increasing variability between plants. The model captured (i) the effect of architectural characteristics of each plant on days 20 and 35 (see the comparison between plants 6, 7 and 10 with low angles with horizontal, vs. plant 2 with higher angle), (ii) the competition between plants on day 50, in which tall plants such as plant 2 had a much higher light interception compared with smaller, dominated plants such as plant 3. Hence, light interception on days 20 and 35 might be considered as similar to those of plants in a canopy with a single genotype, while that on day 50 largely depended on the spatial distribution of different genotypes in the greenhouse.

The suite of methods presented here allowed unifying experiments carried out in different seasons, with a common ranking of genotypes for radiation-use efficiency.

We have tested the interest of the method in an experiment with 200 maize lines and by comparing a set of 23 maize lines in two experiments. The latter were performed at contrasting incident PPFDs in order to compare values of RUE. Overall, plants accumulated more biomass in winter/spring compared to autumn (75%, Fig. 7a), with a correlation between genotype performance in autumn and spring, although the ranking of genotypes slightly changed between experiments (genotype x environment interaction, Fig. 7a inset). Light interception also clearly differed between experiments, largely due to a difference in leaf area (55% difference, not shown). In contrast, the relation between intercepted light and biomass was common to both experiments (Fig. 7e). Indeed, the difference in biomass between experiments was entirely accounted for by the difference in intercepted light (12.5 and 23 mol plant⁻¹ in autumn and winter/spring, respectively, Fig. 7b), attributable to higher leaf area development and higher amount of incident light. Hence, the mean RUE of the canopy (slope of the regression between biomass and intercepted PPFD) was common to both experiments. Furthermore, RUEs measured on individual lines closely correlated between the two experiments (Fig. 7f inset), with neither significant effects of experiment nor genotype x experiment interaction. The genotypic difference in RUE was significant, ranging from 7.0 to 11.1 g FW mol⁻¹. Hence, the methods presented here allowed dissecting the differences between two experiments with large differences in biomass into (i) genotypic traits that did not differ between experiments but had a high genetic variability, namely leaf angle and radiation-use efficiency, (ii) environmental differences, essentially incident light, that affected both biomass and leaf area, thereby generating the large differences that were observed between experiments, (iii) plant traits that differed between experiments due to environmental variables, in particular leaf growth.

We have extended our analysis in Exp. 2b to 1600 plants of 200 genotypes, subjected or not to a mild water deficit (Fig. 8). Overall, both biomass and light interception were affected by water deficit, with a high genetic variability in both cases. Estimated RUE had a large genetic variability (from 6.0 to 12 g FW mol⁻¹) and was affected by water deficit by 12% in average. Hence, the change in biomass with water deficit was related to both intercepted light due to

a lower leaf area and to decreased RUE. In both watering scenarios biomass and RUE displayed high heretabilities ($0.7 < h^2 < 0.8$).

Finally, we have checked whether the observed differences in RUE between genotypes were related to gas-exchange measurements. This was performed in the 8 genotypes with highest contrasts in RUE in Exp. 1 under two contrasting water conditions. Tight correlations were observed between whole-plant RUE values and single-leaf net photosynthesis ($r^2 = 0.54$, $P = 0.001$) and stomatal conductance ($r^2 = 0.61$, $P < 0.001$) (Fig. 9), suggesting that RUE estimated at high throughput with the suite of methods presented here could be a surrogate for gas-exchange measurements and vice versa.

Discussion

A characterization of the light received by each plant based on a model rather than by increasing sensor number

Consistent with intuitive observations of shaded zones in a greenhouse, our study shows that steep gradients of light availability occur over distances smaller than 1m, resulting in differences in incident PPFD by up to 10% between neighbouring plants. Hence, we confirm the large spatial variability of light in greenhouses (Stanhill *et al.*, 1973; Kozai & Kimura, 1977), but also provide a method for mapping it with high spatial resolution. If characterized directly with sensors, this mapping would need at least one sensor per m², i.e. hundreds of sensors technically difficult to maintain and calibrate. The method presented here avoids this problem by accurately simulating the incident light received by each plant at any time of the year. It has the advantage of having a spatial definition of tens of centimetres and of estimating the respective amounts of direct and diffuse PPFD received by plants, which can bias the calculation of intercepted light because of the high proportion of diffuse light in greenhouses (Sinclair *et al.*, 1992; Sinclair & Muchow, 1999). Finally, this method is rapid (the time for taking the 169 images was 4 hours, and the computing time was less than 2 hours). It can be used in any greenhouse regardless of the presence of a phenotyping platform, and is valid as long as the structures of the greenhouse are not changed, and as obstacles to light do not change with time of the year (e.g. a deciduous tree near the greenhouse).

A striking result of our study was the relatively low fraction of total daily transmitted light (ca. 30%) compared with other studies that state light transmissions ranging between 0.46 and 0.84% (Niinemets & Keenan, 2012). This discrepancy is probably due to the fact that most of studies measuring light transmission in greenhouses report values based on midday measurements with sun beams close to the vertical, thereby overestimating transmission (Niinemets & Keenan, 2012).

Dissecting biomass accumulation allows identification of components with high repeatability and genetic variation.

We have shown that coupling a 3D reconstruction method to a structure-function model together with a fine characterisation of environmental conditions allows estimation of light

interception and RUE of thousands of plants with good heritabilities. The suite of methods presented here allowed dissection of biomass into (i) traits that are repeatable between experiments such as RUE or leaf angles, which have a large genetic variability (Mickelson *et al.*, 2002; Tian *et al.*, 2011; [Ku et al., 2012](#)) and can therefore be considered as intrinsic to each genotypes, (ii) traits that are highly dependent on environmental conditions such as the change with time of intercepted light.

In the results presented in this work, RUE values ranged from 7.0 to 11 g FW mol⁻¹. If expressed in a dry weight basis (considering an average water content of 90%) and per unit of light energy (MJ), our estimates range from 3 to 5 g DW MJ⁻¹. These RUE values, although relatively high compared with field measurements, are often reported in plants grown in controlled environments (Hammer & Vanderlip, 1989). This high estimates of RUE can be related to the high proportion of diffuse radiation inherent to greenhouses or chamber structures ([Sinclair et al., 1992](#); [Sinclair & Muchow, 1999](#)). In addition, the lower daily incident radiation in the greenhouse compared with the field may induce high values of RUE through a greater photosynthetic efficiency and compensation mechanisms (Baille *et al.*, 2006). RUE values observed in this work are in accordance with other studies in maize if expressed in a dry weight basis ([Otegui et al., 1995](#); [Lindquist et al., 2005](#); [Louarn et al., 2008](#); [Rattalino Edreira & Otegui, 2012](#)). Proper measurements of plant dry biomass would be needed to compare with available field data. Furthermore, partitioning of biomass to roots and maintenance costs associated to respiration can be an important source of variation in RUE. Such measurements are not easily compatible with the throughput of 1680 plants presented in this study, so only the resulting RUEs can be estimated at this throughput.

Although genetic improvement of RUE has been suggested as a way to increase yield potential in major crops ([Zhu et al., 2010](#); [Reynolds et al., 2011](#)), few studies have explored its genetic variability probably given to the technical difficulties to study this trait (Acreche *et al.*, 2009; Narayanan *et al.*, 2013; [Koester et al., 2016](#)). Other works have shown the potential of 3D reconstruction methods coupled to light distribution algorithms (radiosity or ray tracing) to asses photosynthesis in plant canopies ([Prieto et al., 2012](#); [Song et al., 2013](#); [Pound et al., 2014](#); [Burgess et al., 2015](#)). These methods rely on highly-realistic surface-

based plant reconstructions which require high number of images (ca. 35-65 per plant in complex canopies) or plant digitisations that are difficult to automatize in the context of high-throughput phenotyping. Conversely, our method, uses 3D point clouds that require less reconstruction steps (i.e. binarisation and projection), is easy to automatize with standard image analysis library (openCV), and can be obtained with a limited set of images (from 3 to 12). Although precision in reconstruction is lower compared with the techniques described above, our method can accommodate a certain level of error in the 3D reconstructed plant provided that leaf area estimates are precise enough. Indeed, accurate estimates of the total plant leaf area are easy to obtain from a set of binarised 2D images (Supporting Information Fig. S2). (Golzarian *et al.*, 2011; Hartmann *et al.*, 2011). Another advantage of RATP is that uses a statistical approach that avoids difficulties relating to relative positions of leaves belonging to neighbouring plants. Indeed, the respective positions of leaves may change from one day to another. This is the main reason why we have adopted a probabilistic approach with the RATP model, rather than an explicit description of beam intersecting virtual leaves.

The facts that RUE was highly heritable, repeatable between experiments with different incident light (but similar soil water or nutrient status), and correlated with gas-exchange measurements suggests that our measurement of RUE can have a great value for exploring the genetic variability of a surrogate of canopy photosynthesis at high-throughput in large collections of genotypes, which is a topic of growing interest (Slattery & Ort, 2015; Koester *et al.*, 2016). This method may also allow exploring the change in RUE with environmental conditions by subjecting collections of genotypes to a range of water or nitrogen status, known to largely affect RUE (Teixeira *et al.*, 2014), of CO₂ concentration (Hui *et al.*, 2001; Sakai *et al.*, 2006), or of temperature (Louarn *et al.*, 2008). Finally, using the genotypic values of RUE estimated here in a crop model will allow examining its consequences on yield of a large number of genotypes in a variety of climatic scenarios (Boote *et al.*, 2013; Parent & Tardieu, 2014).

However, we are aware of several methodological difficulties associated with the approach presented here. The first of them is that RUE is the result of a calculation that takes light interception into account. Hence, any error in the estimation of intercepted light results in an error in RUE. The method has been tested successfully in plants with relatively simple

architecture such as maize, but serious errors in the calculation of light interception can occur in plants with complex architecture such as rapeseed, with a high level of occlusion (i.e. in which many leaves are hidden by other leaves). The choice of voxel size can be associated with inaccurate results (Combes *et al.*, 2008) and needs to be adjusted depending on the species and the target variable of study. Indeed, it results from an optimization between (i) an adequate representation of gaps in an open canopy and (ii) conforming to Beer-Lambert assumptions within the voxel.

Other difficulties are associated with methodological choices. If the primary objective is to analyse the genetic control of leaf growth and plant architecture, one tends to use images taken during the night to minimize the change with time of leaf angles or shape due to epinasty (Greenham *et al.*, 2015) or leaf rolling (Hay *et al.*, 2000; Sirault *et al.*, 2015), especially under water deficit. However, this choice can bias the calculation of light interception. Conversely, the use of day-time measurements results in more accurate estimation of light interception but decrease the heritability of measurements of leaf area or angles due to leaf movements or rolling during the day. In the same way, a full characterization of light interception would require that plants are organized in micro-canopies of about 10 plants sharing a common genotype. However, this considerably decreases the number of genotypes studied per experiment, thereby impeding genome-wide association studies that require at least 250-300 genotypes (Beavis, 1998; Malosetti *et al.*, 2013). We show here that working with single plants surrounded by plants of different genotypes can provide good results until plants of different genotypes shade each other (Fig. 7), but some traits such as the vertical distribution of light interception in the canopy cannot be analysed with this design, although it can have an appreciable effect on light interception (Moreau *et al.*, 2012; Sadras *et al.*, 2012). None of these points question the method itself, but rather the protocol of the experiments using the method.

Whereas the method for light mapping can be easily applied in any greenhouse regardless of the presence of a phenotyping platform, the light interception routine is only accessible to platforms equipped with 3D imaging of individual plants, thereby limiting its diffusion. Its main interest is to fill a gap in photosynthesis research, namely the high throughput estimation of light interception and RUE in view of genetic analyses, rather than to be widely distributed in hundreds of platforms.

Conclusion

We believe that the suite of methods proposed here may have a significant impact on future studies of canopy photosynthesis because of it is compatible with the necessary throughput for genetic analyses and because it allows dissecting the genetic variability of biomass accumulation into different traits that have each their genetic architecture. Field-estimations of intercepted light based on field-based imaging or spectral techniques (Comar *et al.*, 2012; Sankaran *et al.*, 2015) will still be necessary, but they can be combined with genotypic values of RUE and leaf angles estimated in the platform, thereby avoiding the time-consuming step of sequential destructive measurements of plant biomass.

Acknowledgements

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Author Contributions

L. C-B., F.T. and C.W. planned and designed the research, L. C-B., B.S performed experiments, L. C-B., C.F. and N.B. analysed data and L. C-B., C.F. and F.T. wrote the manuscript.

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Figure Captions

Figure 1. Hemispheric images of the greenhouse seen from below at a given x-y position, superimposed to the sun paths (yellow lines) during (a) winter and (c) summer solstices and (b) spring and (d) autumn equinoxes. Time courses of the fraction of transmitted direct light at three different positions (red, green and black lines) in the greenhouse at four different dates (e-h). Grey dashed lines represent the average greenhouse transmission value. Maps of the fraction of transmitted direct light in the greenhouse at the same dates (i-l). Black, red and green dots represent the three studied positions in the greenhouse. The black arrow represents the geographical North.

Figure 2. Schematic representation of the method for estimating the local PPFD reaching each x-y position in the greenhouse using daily direct light maps (a), a diffuse light map (b) and the local amount of PPFD (direct + diffuse) light map (c).

Figure 3. Comparison of measured and estimated available PPFD. (a) Bar plots represent the comparison between measured and estimated weekly PPFD at each of the six positions in the greenhouse equipped with light sensors. (b) Time courses of measured and estimated PPFD with an hourly basis at the positions of the six light sensors.

Figure 4. Step-by step method to extract mean leaf angles from a multi-view set of *Zea mays* plant images (a), side image selection from top image (b), binarization (c), skeletonization (d), identification of 50-pixel elements and calculation of angles for each element (e,f). The output of calculations is presented for three maize lines with contrasting architectures. (g) Time course of mean leaf angle as a function of thermal time ($d_{20^{\circ}\text{C}}$) after sowing, autumn experiment (h). Values are the mean \pm SD of 5 replicates.

Figure 5. Canopy structure *Zea mays* plants in the greenhouse superimposed to a three-dimensional array of voxel $0.20\text{ m} \times 0.20\text{ m} \times 0.20\text{ m}$ (a). 3D representation of the grid corresponding to the 1680 plant in the greenhouse (b). Each volume represents a voxel, with a size proportional to the leaf area inside the voxel and a colour representing the dominant leaf angle class. Dark blue 60° , pale blue 53° , green 47° , orange 42° , red 36° .

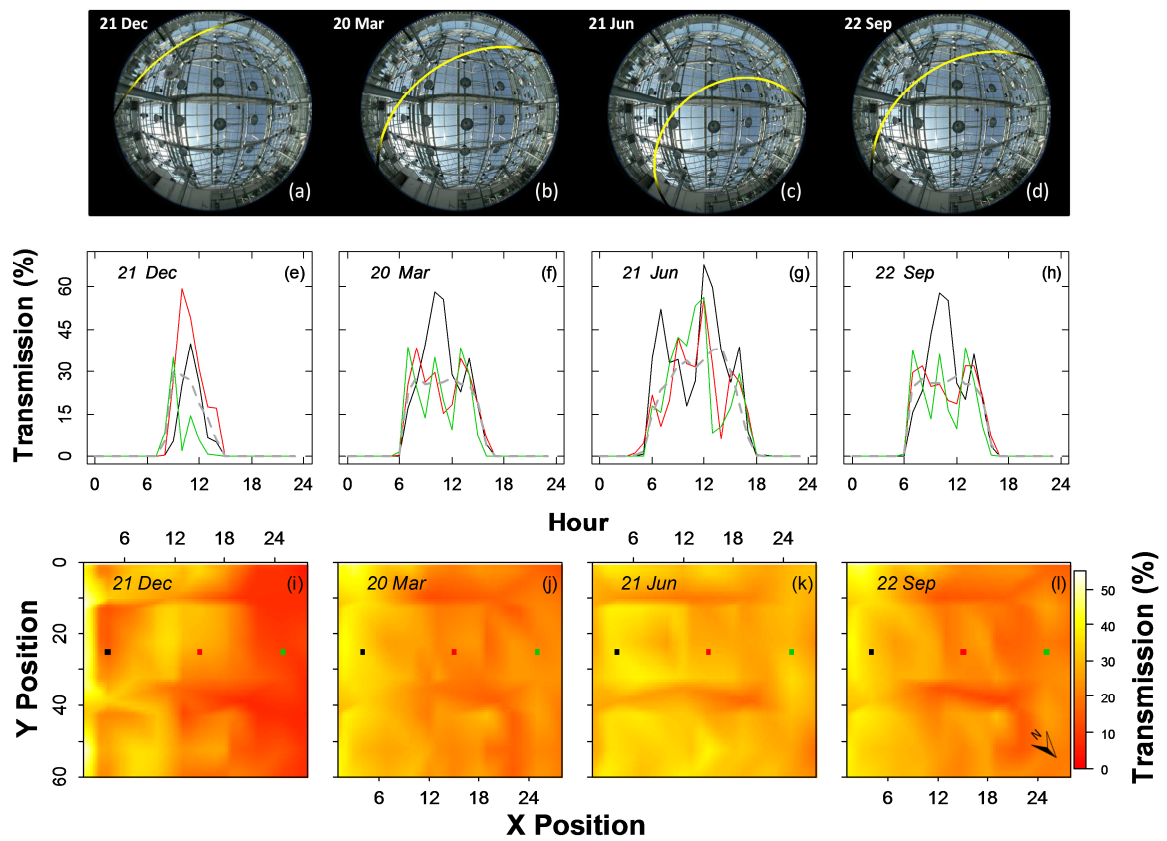
Figure 6. 3D representation of 10 contiguous *Zea mays* plants in the greenhouse at 20 (a), 35 (b) and 50 (c) days after sowing. (d ,e, f) Bar plots representing daily light interception per plant obtained with the RATP model for the plants depicted in Figures a, b and c.

Figure 7. Time courses of biomass accumulation (a) and intercepted PPFD (c), and biomass accumulation of *Zea mays* as a function of intercepted PPFD (d) in two experiments in autumn and winter-spring. Insets in panels a, c and e present the comparison of biomass (b), intercepted PPFD (d) and RUE (f) between experiments. Values are the mean \pm SD of 115 and 92 replicates for autumn and winter experiments, respectively.

Figure 8. Histograms showing variation in cumulated intercepted PPFD (a) and biomass (b) per plant in *Zea mays* plants growing in Exp 2. The relationship between total intercepted PPFD per plant and total cumulated biomass (c). Red and blue symbols / bars refer to water-deficit and well-watered conditions. Each point represents a plant (n= 1600 (200 genotypes x 2 water scenarios x 4 repetitions))

Figure 9. Relationship between radiation-use efficiency (RUE) and leaf net photosynthesis (a) and leaf stomatal conductance (b) in eight genotypes of *Zea mays* with contrasting RUEs grown under well-water (open symbols) and water-deficit conditions (grey symbols). Each point represents the mean \pm SE of 3 replicates.

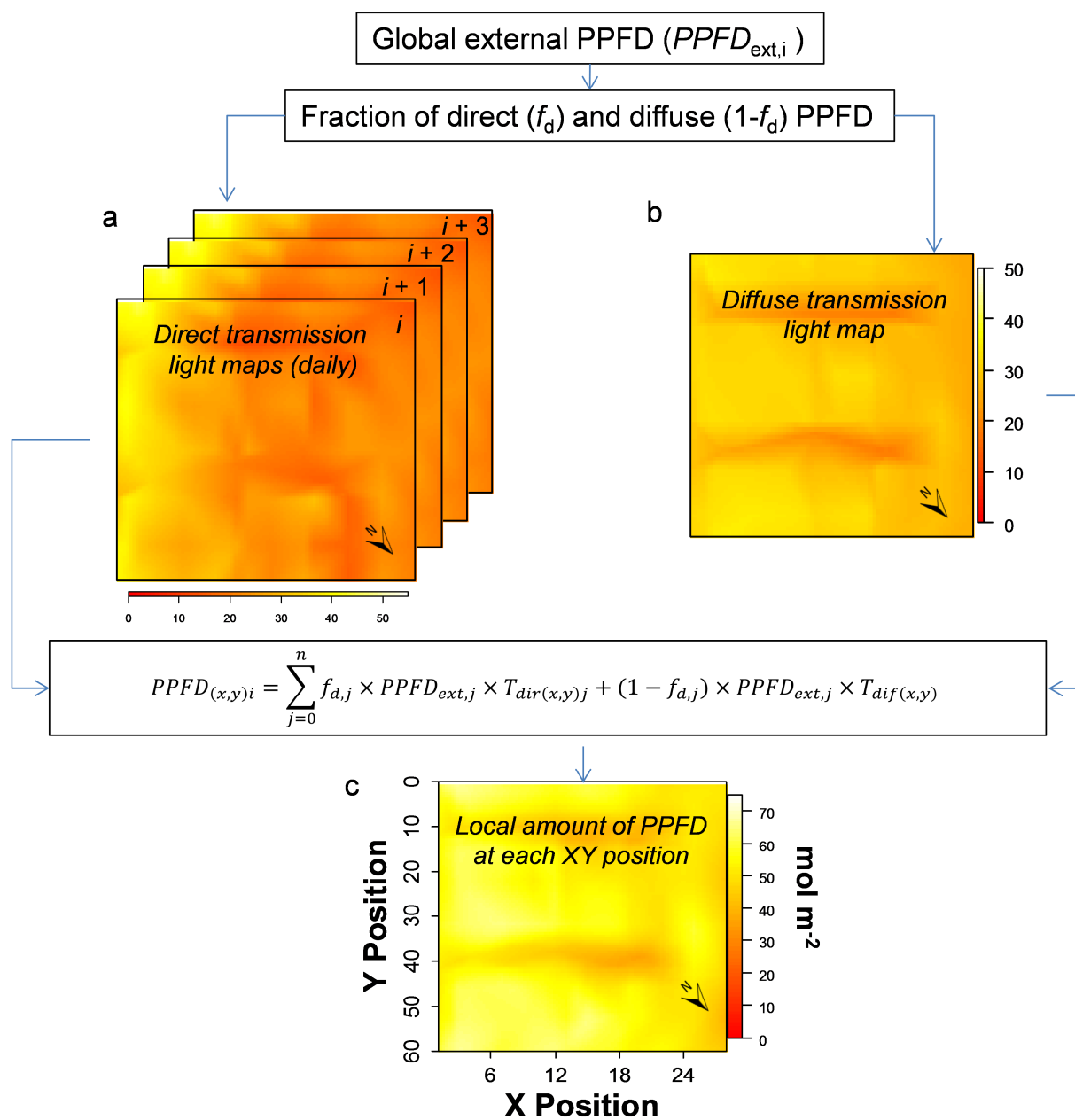
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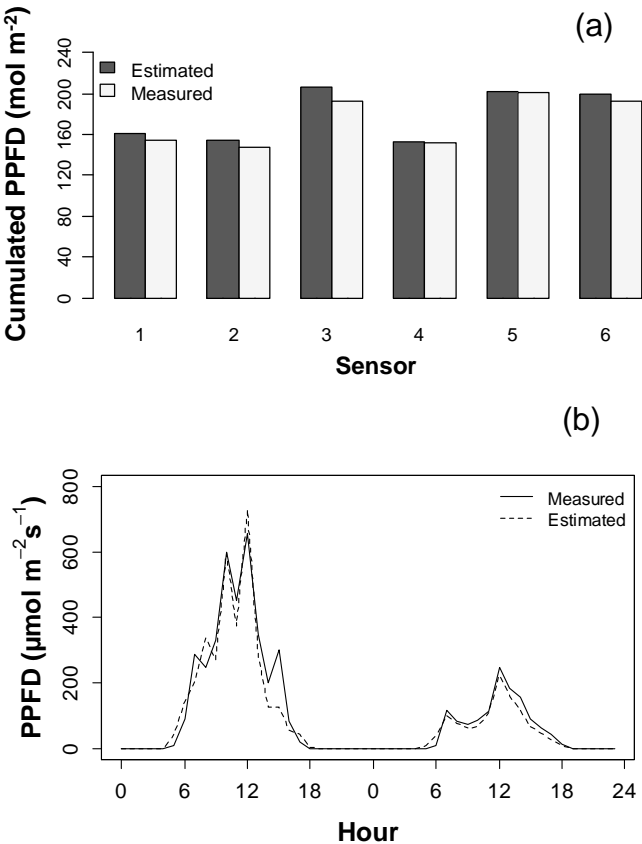
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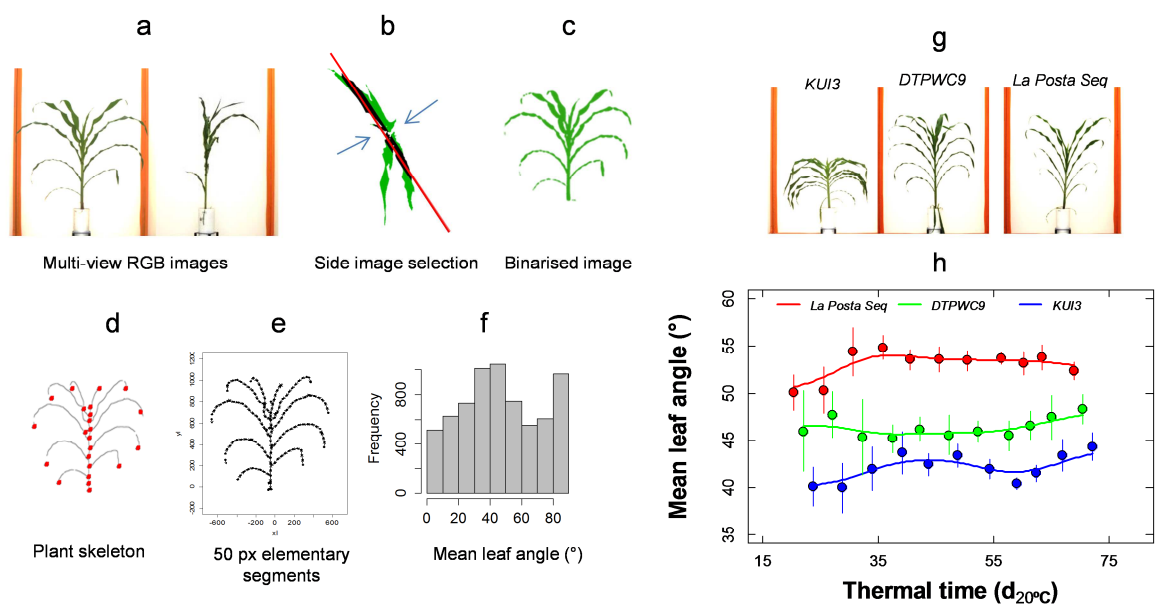
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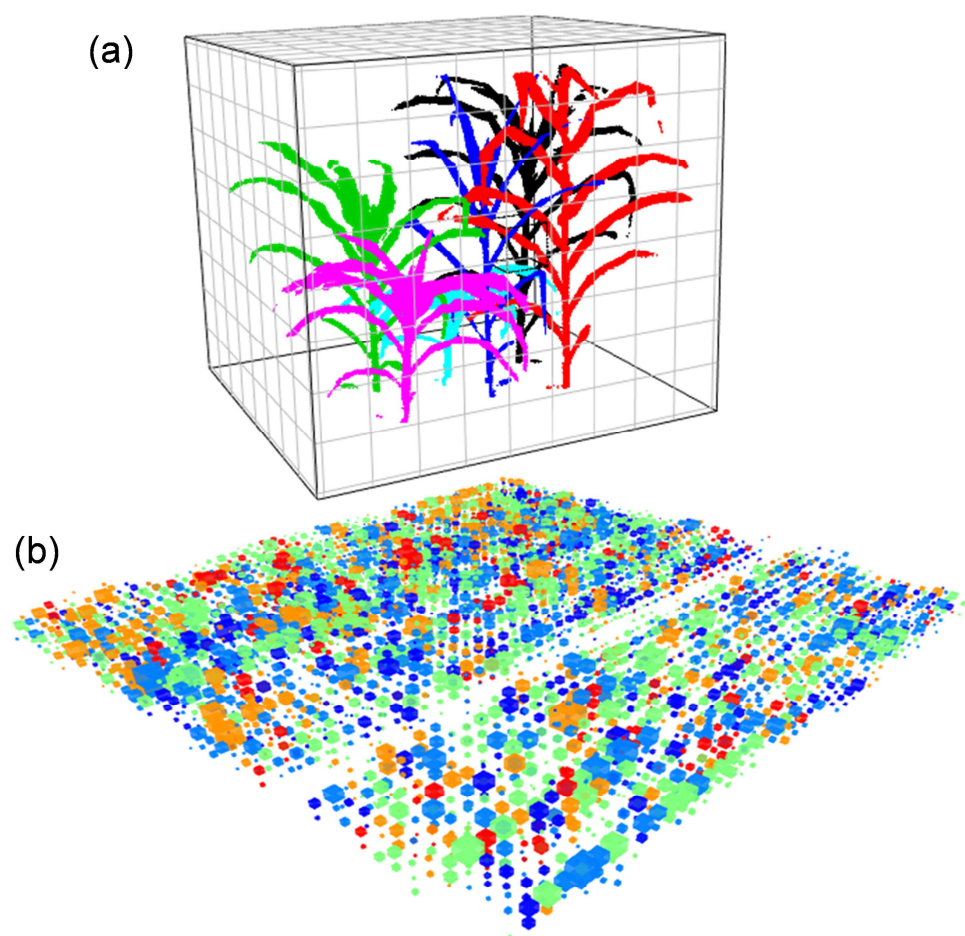


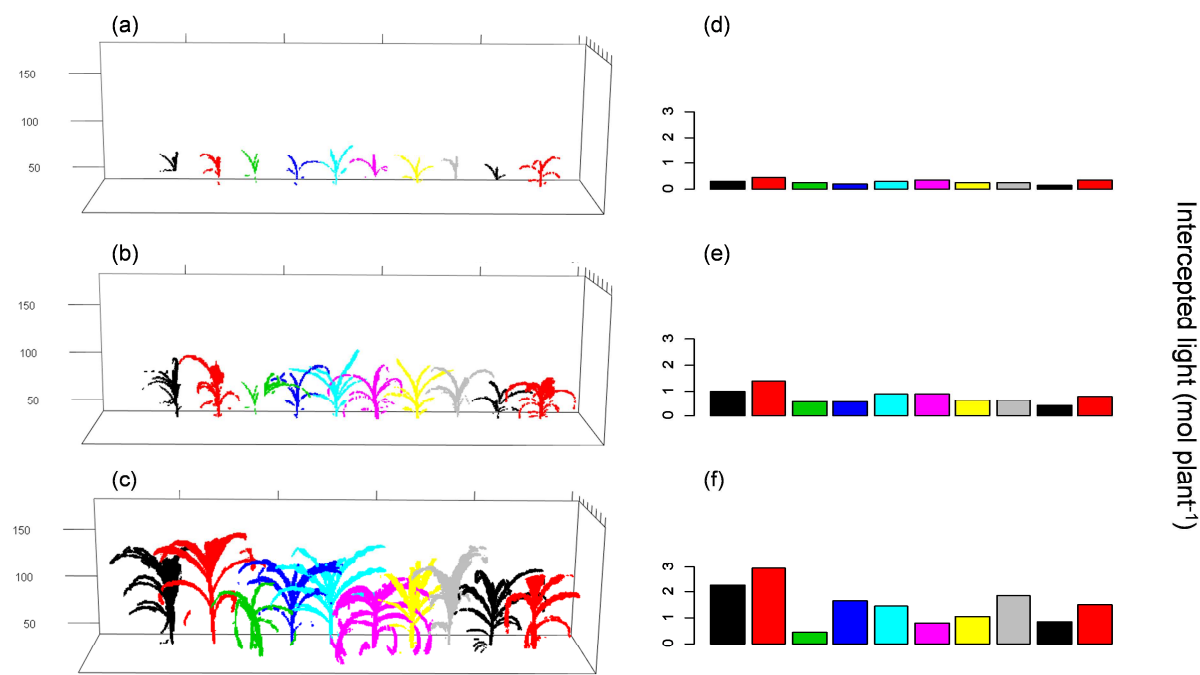
777 Fig. 4

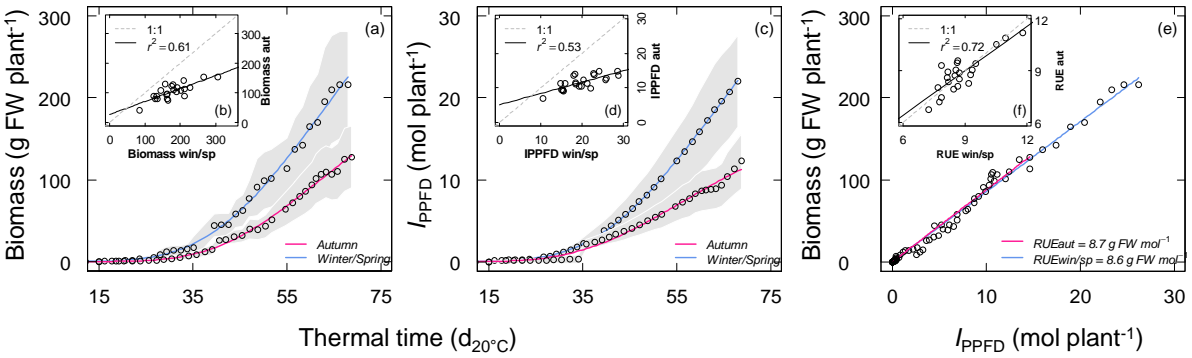


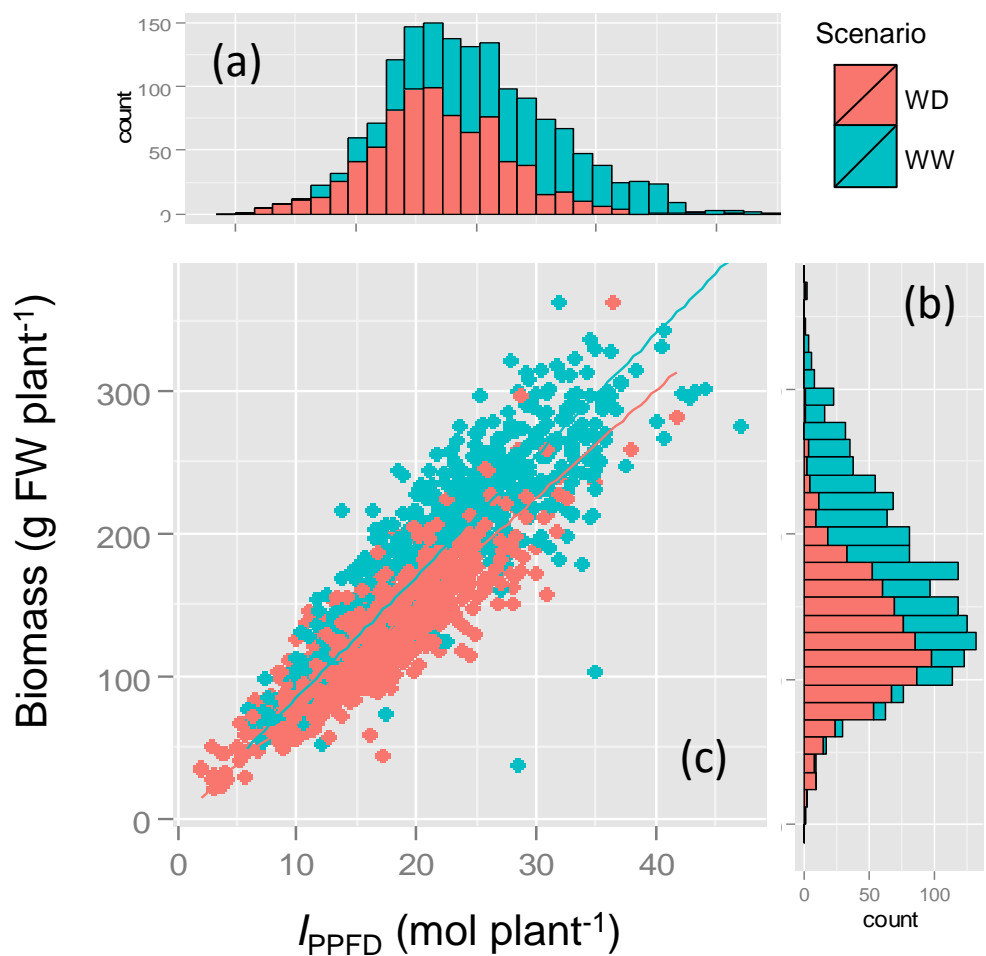
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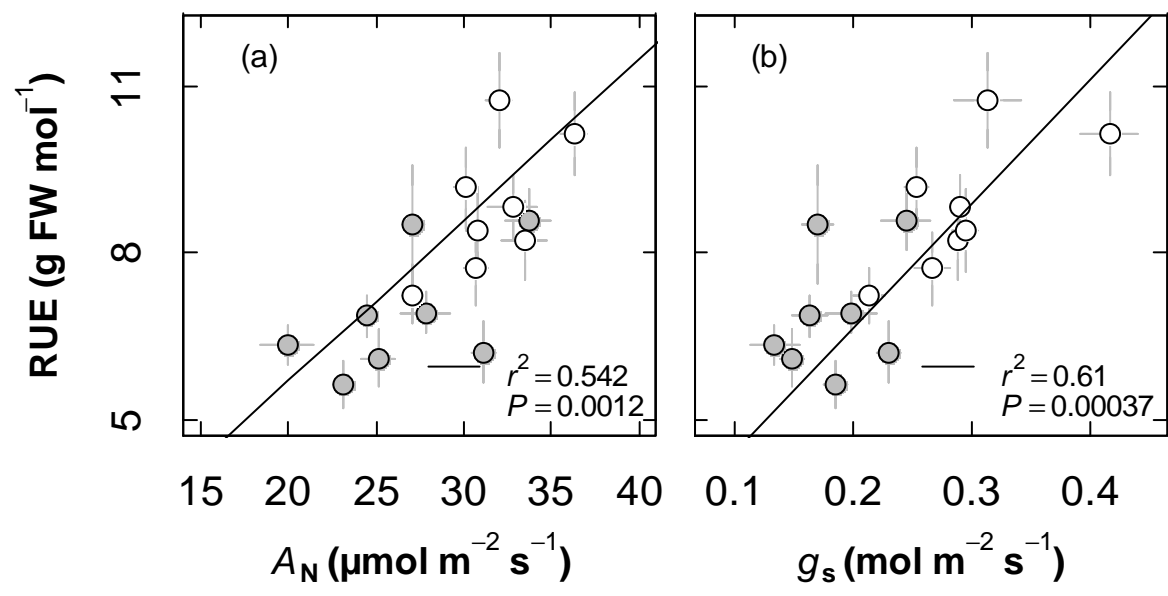
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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1. Pipeline analysis of greenhouse hemispherical images.

Fig. S2. Comparison between measured and predicted leaf area and plant biomass.

Methods S1. Shiny App for Sun Paths and Light transmission calculation.

Table S1. Detailed list of software and packages used in this study.

Video S1. Direct light transmission over a year at the different positions in the greenhouse.

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